

Claims

1. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has an amino acid sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing endochondral bone formation in association with said matrix when implanted in a mammal.

2. A device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

a protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains, each of which has less than about 200 amino acids, in a sequence sufficiently duplicative of the sequence of

COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with said matrix when implanted in a mammal.

3. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
CXXXXLXVXF	DGXWXXX	PXGXXAXY	CXGXCXX	PXXXXXXN
60	70	80	90	100
QXXVXXXNXXXXPXXCCXPXXXXXXLXXXXXXVXLXXYXXMXVXXCX				

wherein each X independently represents an amino acid.

4. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
LXVXF	DGXWXXX	PXGXXAXY	CXGXCXX	PXXXXXXN
60	70	80	90	100
QXXVXXXNXXXXPXXCCXPXXXXXXLXXXXXXVXLXXYXXMXVXXCX				

wherein each X independently represents an amino acid.

5. The device of claim 1 or 2 wherein the sequence comprises:

10	20	30	40	50
CKRHPLYVDFR	DVGWNDWIVAPP	GYHAFYCHG	ECPFPLADH	LNSTNHAIV
RRS K S S L	QE VIS E FD Y	E A AY	MPESMKAS	VI
KE F E K I	DN L	N S	Q ITK F P	TL
Q A S K				
60	70	80	90	100
QTLVNSVNP	GKIPKACCVPT	ELSAISMLY	LDENENV	LKNYQDMVVEGCGCR
SI HAI SEQV EP	A EQMNSLAI	FFNDQDK I	RK EE T DA	H H
RF T S	K DPV V	Y N S	H RN	RS
N S		K	P	E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

6. The device of claim 1 or 2 wherein the sequence comprises:

10 20 30 40 50  
LYVDFRDVGWNDWIVAPPGYHAFYCHGECPPFLADHLNSTNHAIV  
K S S L QE VIS E FD Y E A AY MPESMKAS VI  
F E K I DN L N S Q ITK F P TL  
A S K  
60 70 80 90 100  
QTLVNSVNPKGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGGCGR  
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H  
RF T S K DPV V Y N S H RN RS  
N S K P E

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

7. The device of claim 1 or 2 wherein the sequence comprises:

Vgl 1 10 20 30 40  
CKKRHLYVEFK-DVGWQNWWIAPQGYMANCYGECPYPLTE  
50 60 70  
ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP  
80 90 100  
ISMLFYDNNNDNVVLRHYENMAVDEC GCR

8. The device of claim 1 or 2 wherein the sequence comprises:

DPP 1 10 20 30 40  
CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD  
50 60 70  
HFNSTN--H-AVVQTLVNNNNPGK-VPKACCVPTQLDS  
80 90 100  
VAMLYLNDQSTVVLKNYQEMTVVGCGCR

9. The device of claim 1 or 2 wherein the sequence comprises:

OP1 1 10 20 30 40  
LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFNPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMMVVRACGCH

10. The device of claim 1 or 2 wherein the sequence comprises:

-5  
HQRQA

1 10 20 30 40  
OPL CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPSQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVRACGCH

11. The device of claim 1 or 2 wherein the sequence comprises:

1 10 20 30 40  
CBMP-2a CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD  
50 60 70  
HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA  
80 90 100  
ISMLYLDENEKVVLKNYQDMVVEGCGCR

12. The device of claim 1 or 2 wherein the sequence comprises:

1 10 20 30 40  
CBMP-2b CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD  
50 60 70  
HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA  
80 90 100  
ISMLYLDEYDKVVLKNYQEMVVEGCGCR

13. The device of claim 1 or 2 wherein the sequence comprises:

1 10 20 30 40  
CBMP-3 CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK  
50 60 70  
SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS  
80 90 100  
LSILFFDENKNVVLKVYPNMTVESACR

14. The device of claim 1 or 2 wherein the sequence comprises:

COP1 1 10 20 30 40  
LYVDFQRDVWDDWIAPVDFDAYYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENSTVVLKNYQEMTVVCGCGR

15. The device of claim 1 or 2 wherein the sequence comprises:

COP3 1 10 20 30 40  
LYVDFQRDVWDDWIVAPPGYQAFYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENEKVVALKNYQEMVVEGCGCR

16. The device of claim 1 or 2 wherein the sequence comprises:

COP4 1 10 20 30 40  
LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENEKVVALKNYQEMVVEGCGCR

17. The device of claim 1 or 2 wherein the sequence comprises:

COP5 1 10 20 30 40  
LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD  
50 60 70  
HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80 90 100  
ISMLYLDENEKVVALKNYQEMVVEGCGCR

18. The device of claim 1 or 2 wherein the sequence comprises:

COP7 1 10 20 30 40  
LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD  
50 60 70  
HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80 90 100  
ISMLYLDENEKVVALKNYQEMVVEGCGCR

19. The device of claim 1 or 2 wherein the sequence comprises:

10  
PKHHSQRARKKNKN  
1 10 20 30 40  
COP16 CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD  
50 60 70  
HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80 90 100  
ISMLYLDENEKVVLKNYQEMVVEGCGCR

20. The device of claim 1 or 2 wherein the osteogenics protein comprises a pair of separate polypeptide chains.

21. Osteogenic protein, produced by expression of recombinant DNA in a host cell, capable of inducing endochondral bone formation in association with a matrix when implanted in a mammal.

22. A protein, produced by expression of recombinant DNA in a host cell, comprising one or more polypeptide chains less than about 200 amino acids long in a sequence sufficiently duplicative of the sequence of COP-5 or COP-7 such that said protein is capable of inducing cartilage formation in association with a matrix when implanted in a mammal.

23. The osteogenic protein of claim 21 having an apparent molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel.

24. The osteogenic protein of claim 23 further characterized by being glycosylated.

25. The osteogenic protein of claim 21 having an apparent molecular weight of about 27 kD as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

26. The protein of claim 22 or 25 further characterized by being unglycosylated.

27. The protein of claim 21 or 22 comprising a pair of separate polypeptide chains.

28. The protein of claim 21 or 22 comprising the amino acid sequences:

10            20            30            40            50  
CXXXXLXVXFDXGWWXXXPXXGXXAXYCXGXCXXPXXXXXXNHA<sup>XX</sup>  
60            70            80            90            100  
QXXVXXXNXXXXPXXCCXPXXXXXXLXXXXXXVXLXXYXXMXVXXCX<sup>CX</sup>

wherein each X independently represents an amino acid.

29. The protein of claim 21 or 22 comprising the amino acid sequences:

10            20            30            40            50  
LXVXFDXGWWXXXPXXGXXAXYCXGXCXXPXXXXXXNHA<sup>XX</sup>  
60            70            80            90            100  
QXXVXXXNXXXXPXXCCXPXXXXXXLXXXXXXVXLXXYXXMXVXXCX<sup>CX</sup>  
wherein each X independently represents an amino acid.

30. The protein of claim 21 or 22 comprising the amino acid sequences:

10	20	30	40	50
CKRHPLYVDFRDVGWNDWIVAPPYHAFYCHGECPPFLADHLNSTNHAIV				
RRRS K S S L	QE VIS E FD Y	E A AY MPESMKAS	VI	
KE F E K I	DN L	N S Q	ITK F P	TL
Q A S K				
60	70	80	90	100
QTLVNSVNPKGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGGCR				
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H				
RF T S K DPV V Y N S H RN RS				
N S K P E				

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

31. The protein of claim 21 or 22 comprising the amino acid sequences:

10	20	30	40	50
LYVDFRDVGWNDWIVAPPYHAFYCHGECPPFLADHLNSTNHAIV				
K S S L	QE VIS E FD Y	E A AY MPESMKAS	VI	
F E K I	DN L	N S Q	ITK F P	TL
A S K				
60	70	80	90	100
QTLVNSVNPKGKIPKACCVPTELSAISMLYLDENENVVLKNYQDMVVEGGCR				
SI HAI SEQV EP A EQMNSLAI FFNDQDK I RK EE T DA H H				
RF T S K DPV V Y N S H RN RS				
N S K P E				

wherein, in each position where more than one amino acid is shown, any one of the amino acids shown may be in that position.

32. The protein of claim 21 or 22 comprising the amino acid sequences:

Vgl	1	10	20	30	40
	CKKRHLYVEFK-DVGWQNWIAPQGYMANCYGECPYPLTE				
	50	60	70		
	ILNGSN--H-AILQTLVHSIEPED-IPLPCCVPTKMSP				
	80	90	100		
	ISMLFYDNNNDNVVLRHYENMAVDEC GCR				

33. The protein of claim 21 or 22 comprising the amino acid sequences:

DPP 1 10 20 30 40  
CRRHSLYVDFS-DVGWDDWIVAPLGYDAYYCHGKCPFPLAD  
50 60 70  
HFNSTN--H-AIVQTLVNNNNPGK-VPKACCVPTQLDS  
80 90 100  
VAMLYLNDQSTVVLKNYQEMTVVGCGCR

34. The protein of claim 21 or 22 comprising the amino acid sequence:

OPI 1 10 20 30 40  
LYVSFR-DLGWQDWIIAPEGYAAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVRACGCH

35. The protein of claim 21 or 22 comprising the amino acid sequences:

OPI 1 10 20 30 40  
CKKHELYVSFR-DLGWQDWIIAPEGYAAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPTQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVRACGCH

-5  
HQROA

36. The protein of claim 21 or 22 comprising the amino acid sequences:

CMP-2a 1 10 20 30 40  
CKRHPLYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD  
50 60 70  
HLNSTN--H-AIVQTLVNSVNS-K-IPKACCVPTELSA  
80 90 100  
ISMLYLDENEKVVLKNYQDMVVVEGCGCR

37. The protein of claim 21 or 22 comprising the amino acid sequences:

1 10 20 30 40  
CBMP-2b CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGDCPFPLAD  
50 60 70  
HLNSTN--H-AIVQTLVNSVNS-S-IPKACCVPTELSA  
80 90 100  
ISMLYLDEYDKVVLKNYQEMVVEGCGCR

38. The protein of claim 21 or 22 comprising the amino acid sequences:

1 10 20 30 40  
CBMP-3 CARRYLKVDFA-DIGWSEWIISPKSFDAYYCSGACQFPMPK  
50 60 70  
SLKPSN--H-ATIQSIVRAVGVVPGIPEPCCVPEKMSS  
80 90 100  
LSILFFDENKNVVLKVYPNMTVESCACR

39. The protein of claim 21 or 22 comprising the amino acid sequences:

1 10 20 30 40  
COP1 LYVDFQRDVWDDWIIAPVDFDAYYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENSTVVLKNYQEMTVVCGCR

40. The protein of claim 21 or 22 comprising the amino acid sequences:

1 10 20 30 40  
COP3 LYVDFQRDVWDDWIVAPPGYQAFYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

41. The protein of claim 21 or 22 comprising the amino acid sequences:

1 10 20 30 40  
COP4 LYVDFS-DVGWDDWIVAPPGYQAFYCSGACQFPSAD  
50 60 70  
HFNSTN--H-AVVQTLVNNMNPKG-VPKPCCVPTELSA  
80 90 100  
ISMLYLDENEKVVVLKNYQEMVVEGCGCR

42. The protein of claim 21 or 22 comprising the amino acid sequences:

COP5      1      10      20      30      40  
LYVDFS-DVGWDDWIVAPPGYQAFYCHGECPFPLAD  
50      60      70  
HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80      90      100  
ISMLYLDENEKVVALKNYQEMVVEGCGCR

43. The protein of claim 21 or 22 comprising the amino acid sequences:

COP7      1      10      20      30      40  
LYVDFS-DVGWNDWIVAPPGYHAFYCHGECPFPLAD  
50      60      70  
HLNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80      90      100  
ISMLYLDENEKVVALKNYQEMVVEGCGCR

44. The protein of claim 21 or 22 comprising the amino acid sequences:

COP16      1      10      20      30      40  
-10  
PKHHSSRARKKNKN  
CRRHSLYVDFS-DVGWNDWIVAPPGYQAFYCHGECPFPLAD  
50      60      70  
HFNSTN--H-AVVQTLVNSVNSKI--PKACCVPTELSA  
80      90      100  
ISMLYLDENEKVVALKNYQEMVVEGCGCR

45. The protein of claim 21 or 22 comprising the product of expression of a DNA in a procaryotic cell.

46. A DNA sequence encoding an amino acid sequence sufficiently duplicative of that of the sequence encoded by the gene of Figure 1A such that said encoded sequence induces bone or cartilage formation when implanted in a mammal in association with a matrix.

47. The DNA of claim 46 encoding the same amino acid sequence as the gene set forth in Figure 1A.

48. The DNA sequence of claim 46 encoding:

1 10 20 30 40  
OPI LYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVRACGCH

49. The DNA sequence of claim 46 encoding:

1 10 20 30 40  
OPI CKKHELYVSFR-DLGWQDWIIAPEGYAAYYCEGECAFPLNS  
50 60 70  
YMNATN--H-AIVQTLVHFINPET-VPKPCCAPQLNA  
80 90 100  
ISVLYFDDSSNVILKKYRNMVVRACGCH

50. A cell line engineered to express the protein of claim 21 or 22.

51. The protein of claim 21 having a half maximum bone forming activity of about 20 - 25 ng per 25 mg of implant.

52. A biocompatible, in vivo biodegradable deglycosylated collagenous matrix defining pores of dimensions sufficient to permit influx, proliferation, and differentiation of migratory progenitor cells from the body of a mammal.

53. The matrix of claim 52 comprising close-packed particulate matter having a particle size within the range of 70-850 nm.

54. The matrix of claim 53 wherein said particulate matter has a particle size within the range of 70-420 mm.

55. The matrix of claim 52 defining a shape to span a non-union fracture in said mammal.

56. The matrix of claim 52 comprising demineralized, protein-extracted, deglycosylated, particulate xenogenic bone.

57. The matrix of claim 52 comprising a material selected from the group consisting of hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted, deglycosylated xenogenic bone, and mixtures thereof.

58. An osteogenic device for implantation in a mammal, said device comprising:

a biocompatible, in vivo biodegradable matrix defining pores of a dimension sufficient to permit influx, proliferation and differentiation of migratory progenitor cells from the body of said mammal; and

substantially pure osteogenic protein capable of inducing endochondral bone formation in said mammal disposed in said matrix and accessible to said cells.

59. The device of claim 1, 2, or 58 wherein said matrix comprises close-packed particulate matter having a particle size within the range of 70-850 mm.

60. The device of claim 1, 2, or 58 wherein said particulate matter has a particle size within the range of 70-420 mm.

61. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein-extracted, particulate, allogenic bone.

62. The device of claim 1, 2, or 58 wherein said matrix comprises a material selected from the group consisting of collagen, hydroxyapatite, tricalcium phosphate, polymers comprising lactic acid monomer units, polymers comprising glycolic acid monomer units, demineralized, guanidine-extracted allogenic bone, and mixtures thereof.

63. The device of claim 1, 2, or 58 wherein said matrix is shaped to span a non-union fracture in said mammal.

64. The device of claim 1, 2, or 58 disposed within the marrow cavity of allogenic bone.

65. The device of claim 1, 2, or 58 wherein said matrix comprises demineralized, protein extracted, particulate, deglycosylated xenogeneic bone.

66. The device of claim 65 wherein said matrix is treated with a protease.

67. The device of claim 58 wherein said osteogenic protein is unglycosylated. <sup>ns</sup>

68. The device of claim 67 wherein said osteogenic protein has an apparent molecular weight of about 27 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

69. The device of claim 58 wherein said osteogenic protein is glycosylated. <sup>rs</sup>

70. The device of claim 69 wherein said osteogenic protein has an apparent molecular weight of about 30 kD when oxidized as determined by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis. <sup>rs</sup>

71. The device of claim 58 wherein said osteogenic protein comprises a pair of polypeptide chains.

72. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 14 kD and the other has an apparent molecular weight of about 16 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis. <sup>rs</sup>

73. The device of claim 71 wherein one chain of said pair of polypeptide chains has an apparent molecular weight of about 16 kD and the other has an apparent molecular weight of about 18 kD, both as determined after reduction by comparison to molecular weight standards in SDS-polyacrylamide gel electrophoresis.

74. The device of claim 58 wherein said osteogenic protein has the approximate amino acid composition set forth below:

Amino acid <u>residue</u>	Rel. no. <u>res./molec.</u>	Amino acid <u>residue</u>	Rel. no. <u>res./molec.</u>
Aspartic acid/	22	Tyrosine	11
Asparagine		Valine	14
Glutamic acid/	24	Methionine	3
Glutamine		Cysteine	16
Serine	24	Isoleucine	15
Glycine	29	Leucine	15
Histidine	5	Proline	14
Arginine	13	Phenylalanine	7
Threonine	11	Tryptophan	ND
Alanine	18		
Lysine	12		

75. The device of claim 58 wherein said osteogenic protein comprises the amino acid sequence:

VPKPCCAPT

76. The device of claim 1 or 58 wherein the half maximum bone inducing activity of said protein is 0.8 to 1.0 ng per mg of said matrix.

77. A method of inducing local cartilage or bone formation in a mammal comprising the step of implanting the device of claim 1, 2, or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

78. A method of inducing endochondral bone formation in a mammal comprising the step of implanting the device of claim 1 or 58 in said mammal at a locus accessible to migratory progenitor cells of said mammal.

79. A method of inducing endochondral bone formation in a non-union fracture in a mammal comprising the step of implanting in the fracture in said mammal the device of claim 63.

80. Antibodies reactive with an epitope of the protein of claim 21 or 22.